

SUBJECT: Abbreviated Risk Assessment for J15-31

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***** [REDACTED] *****

SUMMARY

There is low risk associated with the use of the intergeneric [REDACTED] [REDACTED] as there are low potential hazards to human health and the environment from the microorganism and low exposures to workers, the general population, and the environment with this closed system fermentation. The parental microorganism has a long history of safe use and the genetic modifications which consist of the introduction of [REDACTED] [REDACTED] do not pose human health or ecological hazards.

I. INTRODUCTION

The Agency has received a Microbial Commercial Activity Notice (MCAN) from Danisco US, Inc. (operating as DuPont Industrial Biosciences) for an intergeneric *Saccharomyces cerevisiae* strain that has [REDACTED]. In

a previous submission [REDACTED] the recipient *S. cerevisiae* strain was modified by the introduction of a [REDACTED]. In the current submission, a [REDACTED] resulting in the new submission strain [REDACTED]. The parental strain is *S. cerevisiae* [REDACTED]. The genetic modifications allows for [REDACTED] that can then be fermented into ethanol by the microorganism. The production microorganism, strain [REDACTED] will thus be used for production of fuel ethanol from grain.

Although *S. cerevisiae* is one of the ten microorganisms eligible for the 5(h)4 Tiered Exemptions from MCAN reporting, the company has chosen to submit this strain for an MCAN review because [REDACTED]

II. PRODUCTION VOLUME

The proposed production volumes for years 1, 2, and 3 are given in the following table.

Year	# of Batches	Dry Cell Weight (kg) per Batch	Total Dry Cell Mass (kg)	Total Wet Cell Mass Fermentation Broth (kg)	Total Wet Cell Mass Concentrated Cream (kg)	Total Active Dry Yeast Product (kg)
1	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
2	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
3	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

Using bench scale experiments, the company estimates [REDACTED]

III. TAXONOMY

The recipient microorganism is *Saccharomyces cerevisiae* strain [REDACTED], data on 18S rDNA sequencing performed by the company was submitted to confirm the identity as *S. cerevisiae*. The identity of the parental strain is accepted as being *S. cerevisiae* (Segal, 2015b).

IV. HISTORY OF SAFE USE

Saccharomyces cerevisiae has an extensive history of safe use in the area of food processing. Also known as baker's yeast or brewer's yeast, this organism has been used for centuries as a leavening for bread and as a fermenter of alcoholic beverages. The risk assessment of *S. cerevisiae* for the 5(h)(4) Tiered Exemptions Final Risk Assessment for *S. cerevisiae* (<http://www.epa.gov/opt/biotech/pubs/pdf/fra002.pdf>) concluded that this yeast presents low hazards to human health and to the environment. EPA has reviewed this recipient microorganism in a number of recent MCANs: [REDACTED]

V. CONSTRUCT ANALYSIS

A. Construction of the Submission Microorganism

The construction of the precursor strain [REDACTED]

[REDACTED]

No antibiotic resistance markers remained in the submission microorganism, strain [REDACTED]

[REDACTED]

[REDACTED]

All of the modifications were performed in such a way that no bacterial vector sequences remain in the submission strain. No introduced antibiotic resistance markers remain in the submission. This was verified by characterization of the strain by Southern blot analyses and polymerase chain reaction (PCR).

The genetic modifications enable the submission strain to [REDACTED]

[REDACTED] Wild-type *S. cerevisiae* does not [REDACTED]

B. Potential Hazards of the Genetic Modifications

There is low hazard associated with the introduced [REDACTED] gene to create the submission strain of [REDACTED]. The introduced [REDACTED]

[REDACTED] have a long history of safe use in the food industry. [REDACTED] is common in microorganisms and merely enables the production organism to convert [REDACTED] which can then be fermented into ethanol.

There is low hazard associated with the introduced [REDACTED]

[REDACTED] and is also found in humans and other mammals. The donor microorganism, [REDACTED], is ubiquitous in the environment in soils, water, and vegetation. It has a long history of safe use for [REDACTED].

The introduced genes were both stably inserted into the chromosome which means they are unlikely to horizontally transfer to other microorganisms in the environment if the submission microorganism was inadvertently released from the manufacturing facility or ethanol production plants. There is low concern for horizontal gene transfer, and there would be low concern even if the genes were to be transferred.

VI. HUMAN HEALTH HAZARDS

The concern for human health effects associated with the recipient microorganism is low (Ward, 2015). The recipient strain for the MCAN submission is *Saccharomyces cerevisiae* which has extensive history of safe use. *Saccharomyces cerevisiae* is non-pathogenic and non-toxic.

The introduced genetic material also does not present health-related concerns. [REDACTED]

[REDACTED]. Since the company uses personal protective equipment (lab coats, safety glasses, latex or nitrile gloves, uniforms, and respirators) during manufacturer, there is low concern for exposure and allergy due to the submission microorganism.

Since there are no introduced antibiotic resistance markers in the final organism, there is low concern for antibiotic resistance genes spreading in the environment.

VII. ECOLOGICAL HAZARDS

There are low ecological hazard concerns for the recipient microorganism, *S. cerevisiae* (Muneer, 2015). The recipient microorganism does not pose any pathogenicity/toxicity concerns to plants or animals. It is a benign yeast that is ubiquitous in the environment. The risk assessment of *S. cerevisiae* for the 5(h)4 Tiered Exemption stated that there are low ecological hazards associated with this microorganism.

The introduced [REDACTED] genes do not pose any concerns for pathogenicity/toxicity of the submission microorganism. It merely enables the yeast, [REDACTED], thus enabling its use on grains in ethanol production. Although the strain may survive if inadvertently released into the environment, there would be no ecological concerns (Muneer, 2015).

VIII. INTEGRATED RISK ASSESSMENT

There is low risk to human health and the environment associated with the production of and use of [REDACTED] containing the intergeneric genes encoding [REDACTED]. The recipient microorganism is not pathogenic to humans, other animals, or to plants. It has a long history of safe use and is ubiquitous in the environment. The [REDACTED]s are common in many microorganisms and do not pose human health or ecological concerns. [REDACTED] which is subsequently fermented into ethanol. The genes are stably inserted into the chromosome which reduces the potential for horizontal gene transfer if the microorganisms were inadvertently released in the environment. There are no bacterial vector sequences remaining in the production microorganism. The production microorganism does not contain any introduced antibiotic resistance marker genes. Thus, there is low risk to human health and the environment with the manufacture and use of this *Saccharomyces cerevisiae* strain [REDACTED]

REFERENCES

[REDACTED]

[REDACTED]

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